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Precursor/product antiport in bacteria

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Summary

Many microorganisms metabolize their substrates (precursors) only partially and excrete the products of the metabolism into the medium. Although uptake of precursor and exit of product can proceed as two independent steps, there is increasing evidence that these processes are often linked and that transport is facilitated by a single antiport mechanism. Features of antiport mechanisms and advantages for the organism of catalysing precursor/product antiport will be illustrated by discussing a number of well-characterized systems. Based on precursor-product conversion stoichiometries, structural relatedness between precursors and products, and energetic and kinetic considerations, new examples of antiport systems will be proposed.

Introduction

Solute transport systems in bacteria can be classified into three categories according to the mode of energy coupling (Konings *et al.*, 1989): (i) primary transport systems utilize chemical or light energy to translocate a molecule across the cytoplasmic membrane; (ii) secondary transport systems utilize electrochemical energy for solute translocation; (iii) group translocation systems couple the translocation to a chemical modification of the solute. For the secondary solute transport systems the electrochemical gradient for protons and/or sodium ions most often provides the driving force for transport. In recent years, however, a number of transport systems have been described in which the inward movement of precursor is directly coupled to the outward movement of product (precursor/product antiport). Depending on the charge of the substrates and the stoichiometry of the antiport, the process can be independent of the proton-motive force (Δp), the antiport can generate metabolic energy in the form of a Δp or the antiport can be partially driven by the Δp. The main goal of this article is to review the evidence for antiport systems of metabolites in bacteria and to give new examples of metabolic pathways in which an antiport mechanism may be operative. These examples could be instructive for further identification of antiport systems in pathways of microbial degradation and fermentation. Cation antiport systems involved in the accumulation and/or extrusion of inorganic cations, like the Na^+/H^+ antiporters, the Ca^{2+}/H^+ antiporter and others, will not be discussed.

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Solute antiport or exchange

Transport systems that mediate proton-coupled solute transport bind the solute and proton at the outer surface of the cytoplasmic membrane, and, following transmembrane translocation, the molecules are released into the cytoplasm (Kaback, 1983; Konings *et al.*, 1989; Poolman *et al.*, 1987b). If the rate of release of the solute from the carrier exceeds the rate of deprotonation and if excess solute is present in the cytoplasm, rebinding and efflux of solute can occur prior to the release of the proton. Under these conditions, the carrier protein catalyses solute/solute exchange (or antiport) instead of solute/H^+ symport. The exchange reaction can be monitored by differential labelling of the solutes in the external and internal compartments. The exchange of identical solutes is termed 'homologous exchange' or 'solute self-exchange'. Solute exchange can be homologous but also heterologous (with different solutes internally and externally), and can proceed independently of the magnitude and polarity of the Δp.

Although mechanistically similar to the antiport reactions described below, the homologous exchange does not contribute to net movement of a solute. In contrast, heterologous exchange results in the accumulation of a solute (precursor) in the cytoplasm concomitant with the excretion of another solute (product) into the external medium. This type of transport, also referred to as 'facilitated exchange diffusion', has been known to occur across the inner mitochondrial membrane, the inner membrane of chloroplasts, and the vacuolar membrane of yeasts (Fliege *et al.*, 1978; Flugge and Heldt, 1986; Klingenberg, 1980; McGiven and Klingenberg, 1971; Sato *et al.*, 1984). For instance, ADP enters the mitochondrial matrix only if ATP exits into the cytosol, and vice versa.

Since ATP has one additional negative charge relative to ADP, ATP/ADP exchange is driven not only by the concentration gradients of ATP and ADP but also by the $\Delta\psi$ (Klingenberg, 1980). The inner mitochondrial membrane also contains antiport systems for dicarboxylic acids, e.g. glutamate-aspartate and malate-α-ketoglutarate (LaNoue and Schoolwerth, 1979; Murphy *et al.*, 1979). The combined action of these systems constitutes a cyclic transport pathway called the 'malate–aspartate shuttle'. Besides the eukaryote organelles, an adenine nucleotide exchange system has been demonstrated in the eukaryotic parasite *Rickettsia prowazekii* (Krause *et al.*, 1985; Winkler, 1976). The exchange of ATP for ADP supplies the parasites with a source of metabolic energy at the expense of the host cell.

Pathway intermediates are usually not abundant in the environment of free-living bacteria, which makes antiport mechanisms of restricted value. However, many microorganisms metabolize their substrates only partially and excrete the products of the metabolism into the medium. When the end-products are structurally related to the substrates (precursors), transport of both can be facilitated by the same carrier protein. A further prerequisite for precursor/product antiport is the stoichiometric conversion of precursor into end-product. Below (Table 1), examples will be given which meet the criteria for an antiport mechanism. It is concluded that several antiport systems used by bacteria appear to have evolved in specialized transport mechanisms which only catalyse exchange and not solute/cation symport.

Arginine/ornithine antiport

The arginine deiminase (ADI) pathway is widely distributed among bacteria and serves as sole or additional source of energy, carbon and/or nitrogen in these organisms (Crow and Thomas, 1982; Cunin *et al.*, 1986; Fenske and Kenny, 1976; Poolman *et al.*, 1987a). The ADI pathway includes: (i) arginine deiminase, which catalyses the conversion of arginine into citrulline and ammonia in an essentially irreversible reaction (Cunin *et al.*, 1986); (ii) ornithine carbamoyltransferase, which catalyses the phosphorolysis of citrulline, yielding ornithine and carbamoylphosphate (this step is thermodynamically limiting since the equilibrium of the reaction strongly favours the formation of citrulline ($K \sim 10^5$) (Stalon, 1972; Stalon *et al.*, 1972)); (iii) carbamate kinase, which catalyses the reversible conversion of carbamoylphosphate and ADP into ATP, carbon dioxide and ammonia. In addition to these enzymatic steps, the precursor and products of the ADI pathway have to be translocated across the cytoplasmic membrane (Fig. 1A).

A number of observations have led to the suggestion

Table 1. Precursor/product antiport in bacteria.

Antiporter	Pathway	Organism
Arginine/Ornithine	Arginine deiminase	*Lactococcus lactis* *Streptococcus sanguis* *Streptococcus milleri* *Enterococcus faecalis* *Pseudomonas aeruginosa*
Agmatine/Putrescine	Agmatine deiminase	*Enterococcus faecalis*
Sugar-phosphate/Phosphate	Glycolytic	*Lactococcus lactis* *Escherichia coli* *Staphylococcus aureus*
sn-glycerol 3-phosphate/ Phosphate	Glycolytic	*Escherichia coli*
PEP/Phosphate	Glycolytic	*Salmonella typhimurium*
Phosphate/Phosphate	—	*Streptococcus pyogenes*
Lactose/Galactose	Glycolytic	*Streptococcus thermophilus* *Lactobacillus bulgaricus*
Oxalate/Formate	Oxalate decarboxylation	*Oxalobacter formigenes*
Malate/Lactate	Malolactic fermentation	*Leuconostoc* sp. *Lactobacillus* sp. *Lactococcus* sp. *Pediococcus* sp.
Betaine/Dimethylglycine	Betaine oxidation	*Desulfobacterium* sp. *Eubacterium limosum*

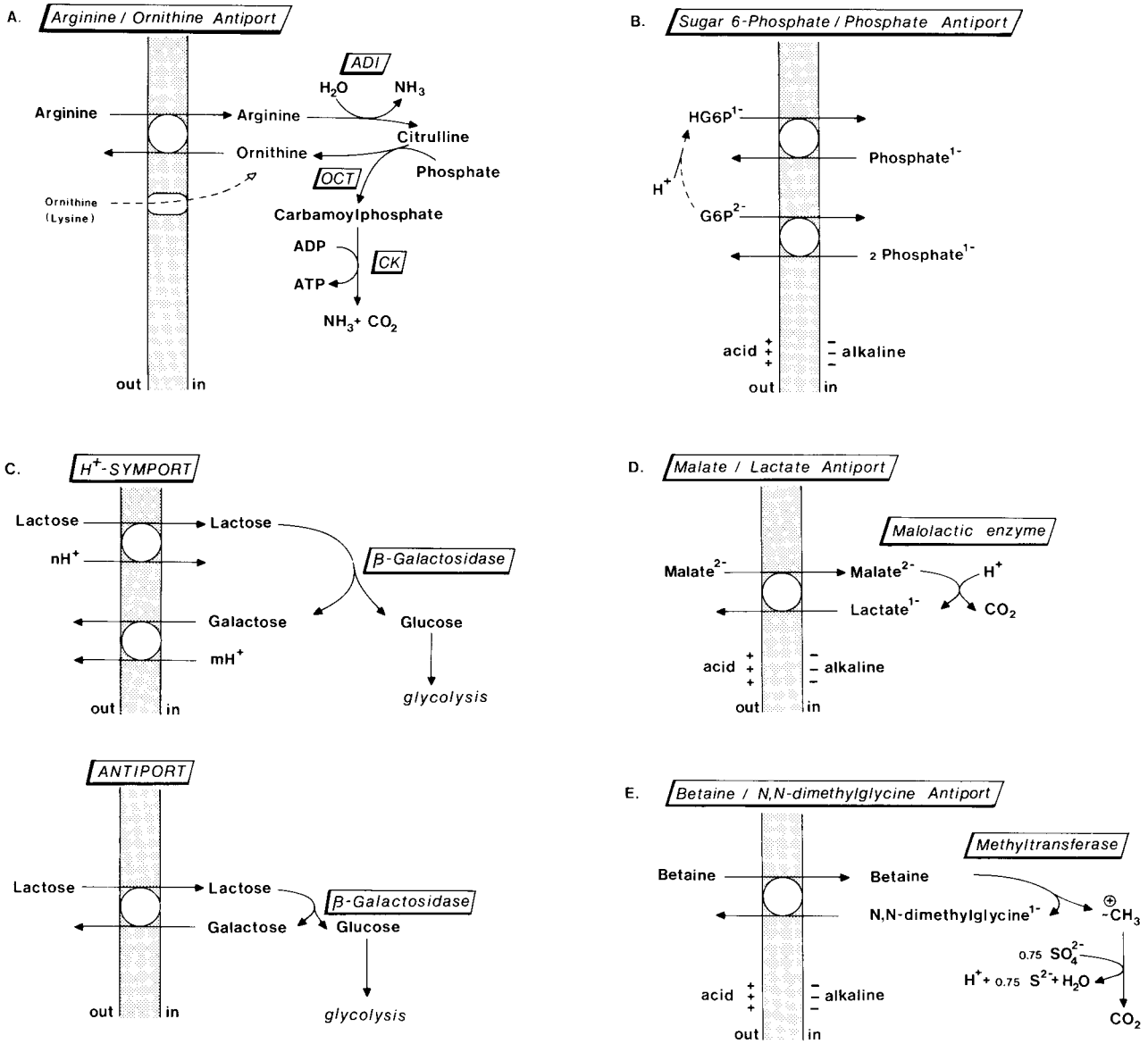

Fig. 1. Bacterial antiport mechanisms.

A. Arginine/ornithine antiport. Accumulation of ornithine (lysine) via the lysine transport system is also depicted in the Figure. ADI, arginine deiminase; OCT, ornithine carbamoyltransferase; CK, carbamate kinase.

B. Sugar 6-phosphate/phosphate antiport. Variable stoichiometry of sugar phosphate/phosphate exchange is indicated.

C. Galactoside/H^+ symport versus lactose/galactose antiport. n and m refer to the stoichiometry of lactose/H^+ and galactose/H^+ symport, respectively.

D. Malate-lactate antiport.

E. Betaine/*N,N*-dimethylglycine antiport. ~CH_3, methyl group coupled to corrinoid protein of the acetyl CoA/CO dehydrogenase pathway (Heythuizen and Hansen, 1989). The electrogenicity of malate/lactate and betaine/*N,N*-dimethylglycine antiport is indicated by the charge of the solutes and the membrane potential (inside negative).

that arginine uptake and ornithine extrusion are facilitated by an antiport mechanism and this has been demonstrated in intact cells, membrane vesicles and proteoliposomes of *Lactococcus lactis* (designated *Streptococcus lactis* in previous papers) (Driessen *et al.*, 1987; Poolman *et al.*, 1987a; Thompson, 1987), in intact cells of *Enterococcus faeacalis* (designated *Streptococcus faecalis* in previous papers), *Streptococcus sanguis* and *Streptococcus milleri* (Poolman *et al.*, 1987a), and in membrane vesicles of an *Escherichia coli* strain containing the arginine transport gene of *Pseudomonas aeruginosa* (H. Verhoogt and D. Haas, unpublished). (i) Many bacteria, including *L. lactis*, possessing the ADI pathway excrete one mole of ornithine per mole of arginine metabolized (Crow and Thomas, 1982; Vander Wauven *et al.*, 1984). (ii) Arginine catabolism by the ADI pathway yields only one ATP per arginine (Cunin *et al.*, 1986). The net gain of metabolic energy, however, will depend on the energetic

costs of arginine uptake and ornithine excretion. Studies of the molar growth yields on arginine in *L. lactis, E. faecalis*, and other bacteria indicated the net formation of one ATP per arginine (Crow and Thomas, 1982; Pandey, 1980), implying that no metabolic energy is needed for the transport processes. (iii) Resting cells of *L. lactis* maintain a high intracellular concentration of ornithine in the absence of (exogenous) metabolic energy (Poolman *et al.*, 1987a; Thompson, 1987). (iv) Arginine uptake rates are maximal when the intracellular ATP concentration and the Δp are low (Poolman *et al.*, 1987a). Based on similar criteria and published data, it can be proposed that arginine/ornithine antiport is also operative in halobacteria (Hartmann *et al.*, 1980), mycoplasma (Schmike *et al.*, 1966) and other bacteria possessing the ADI pathway (Cunin *et al.*, 1986).

Although arginine/ornithine antiport has been demonstrated in intact cells (Poolman *et al.*, 1987a; Thompson, 1987), the most rigorous evidence comes from experiments in which arginine metabolism has been eliminated, i.e. membrane vesicles and proteoliposomes (Driessen *et al.*, 1987). For these studies, membrane preparations were loaded with [^{3}H]-ornithine and diluted into media with or without [^{14}C]-arginine. Rapid uptake of arginine was observed concomitantly with ornithine efflux. Hardly any uptake of arginine occurred when proteoliposomes were not loaded with ornithine, and very little ornithine efflux took place in the absence of arginine externally. Arginine/ornithine antiport was found to be independent of the magnitude and composition of the Δp and proceeded with an apparent stoichiometry of one (Driessen *et al.*, 1987). Consequently, the driving force for arginine uptake in intact cells is supplied by the ornithine and arginine concentration gradients formed during arginine metabolism. Detailed kinetic analysis of arginine/ornithine antiport indicates that the carrier possesses a single substrate-binding site which is present alternately at the inner and outer surface of the cytoplasmic membrane (Driessen *et al.*, 1989a). The exchange reaction catalysed by the antiporter resembles a 'ping-pong' mechanism regarding enzyme kinetics.

Since uptake of arginine and excretion of ornithine via the antiporter are tightly linked, questions arise with respect to initiation of arginine metabolism and replenishing of the ornithine pool when a fraction of the arginine is used for biosynthetic purposes. *L. lactis* has solved the dilemma by taking advantage of a Δp-driven lysine transport system that can accept ornithine with low affinity (Driessen *et al.*, 1989b). More importantly, perhaps, the antiporter catalyses heterologous exchange of arginine and lysine in addition to heterologous exchange of arginine and ornithine. Accumulation of lysine via the Δp-driven transport system in combination with exchange of lysine for arginine via the antiporter results in cyclic transport of lysine and a net accumulation of arginine. In this scheme, a constant level of ornithine can be sustained when part of the arginine is used for biosynthesis.

In apparent conflict with each other are the observations that arginine-uptake rates in intact cells are maximal when intracellular ATP levels and Δp are low, whereas arginine/ornithine antiport in proteoliposomes is not affected by the Δp. The effect of Δp on arginine/ornithine antiport in intact cells can be explained, however, by a feedback mechanism in which the F_0F_1-ATPase (i.e. a back pressure effect of Δp on ATP hydrolysis), ornithine carbamoyltransferase (regulated allosterically by (adenine) nucleotides (Stalon, 1972; Stalon *et al.*, 1972)) and carbamate kinase (which operates close to equilibrium) are involved. As a result, the activity of the ADI pathway is affected by the internal concentration of (adenine) nucleotides. Conditions that lower ATP consumption (Δp is high) decrease the ADI pathway activity whereas conditions that stimulate ATP consumption (Δp is low) increase the activity (Poolman *et al.*, 1987a). The arginine/ornithine antiport activity in intact cells matches the ADI pathway by adjusting the intracellular levels of ornithine and arginine (Poolman *et al.*, 1987a).

Finally, exchange between ornithine (or arginine) and citrulline does not occur in membrane vesicles or in intact cells. This enables the cells to maintain the high intracellular concentrations of citrulline required to drive the thermodynamically unfavourable reaction, catalysed by ornithine carbamoyl-transferase, towards ornithine and carbamoylphosphate (Stalon, 1972).

Agmatine/putrescine antiport

E. faecalis can use agmatine as sole source of energy for growth (Roon and Barker, 1972; Simon and Stalon, 1982). Agmatine is metabolized via the agmatine deiminase (AgmDI) pathway, yielding one mole of putrescine, carbon dioxide and ATP and two moles of ammonia per mole of agmatine consumed. By analogy with arginine/ornithine antiport it has been proposed that agmatine uptake and putrescine excretion are facilitated by an agmatine/putrescine antiport (Poolman *et al*, 1987a). Agmatine/putrescine antiport has been demonstrated in membrane vesicles of *E. faecalis*. The exchange has a stoichiometry of one and the process is not affected by the magnitude and composition of the Δp (Driessen *et al.*, 1988).

Sugar phosphate-phosphate antiport

The evidence for sugar phosphate/phosphate antiport in bacteria has been reviewed recently (Maloney *et al.*, 1990), and only the relevant features of this exchange will be dealt with in this section. The first anion exchange system described in a free-living prokaryote is the sugar-phosphate antiport of *L. lactis* 7962 (Maloney *et al.*, 1984). This

transport system catalyses the homologous and heterologous exchange of phosphate and various sugar 6-phosphates (Ambudkar and Maloney, 1984). The exchange reaction is electroneutral under all conditions tested. To maintain electroneutrality during heterologous exchange, the antiport system translocates phosphate/sugar 6-phosphate with a pH-dependent variable stoichiometry (Fig. 1B). The results indicate that the antiport has specificity for monovalent phosphate and that it selects randomly among the available mono- and divalent sugar 6-phosphates (Ambudkar *et al.*, 1986b). At pH 7.0 (0.9 pH units above the pK_2 of sugar 6-phosphate) the carrier catalyses exchange of two molecules of monovalent phosphate for one molecule of divalent sugar 6-phosphate, whereas at pH 5.2 (0.9 pH units below the pK_2 of sugar 6-phosphate) the exchange corresponds with one molecule of monovalent phosphate for one molecule of monovalent sugar 6-phosphate.

The discovery of sugar 6-phosphate/phosphate antiport in *L. lactis* has led to a re-evaluation and re-examination of anion transport in bacteria. The transport systems for *sn*-glycerol 3-phosphate (GlpT protein) and hexose phosphate (UhpT protein) of *Escherichia coli*, which were thought to be driven by the Δp (Harold, 1977; Leblanc *et al.*, 1980), appear to mediate *sn*-glycerol 3-phosphate/phosphate and sugar 6-phosphate/phosphate exchange, respectively (Ambudkar *et al.*, 1986a; Elvin *et al.*, 1985; Sonna *et al.*, 1988). Similar observations have been made for the phosphoglycerate-phosphoenolpyruvate transport system (PgtP protein (Saier *et al.*, 1975)) of *Salmonella typhimurium* (P. C. Maloney, personal communication) and the sugar phosphate transport system of *Staphylococcus aureus* (Sonna and Maloney, 1988; Mitchell, 1954). Also, these antiport systems were considered to be driven by Δp (Harold, 1977). Although the possibility that some of these systems catalyse sugar phosphate/phosphate exchange in addition to (sugar) phosphate/proton symport cannot be excluded, the effects of Δp could be indirect. For instance, a pH gradient (alkaline inside relative to the outside) may promote exchange of one molecule of divalent sugar 6-phosphate for two molecules of monovalent sugar 6-phosphate, which results in net accumulation of sugar 6-phosphate (Ambudkar *et al.*, 1986b). In this view, the pH gradient is not mechanistically involved in sugar 6-phosphate transport but interferes by substrate (de)protonation. The previously reported electrogenicity of sugar 6-phosphate uptake in membrane vesicles of *E. coli* (Leblanc *et al.*, 1980) could be related to membrane potential-driven phosphate uptake via the Pit system, resulting in the formation of a phosphate concentration gradient that actually drives sugar 6-phosphate uptake (Poolman, 1987; Sonna and Maloney, 1988; Sonna *et al.*, 1988).

The observation that the antiporter catalyses anion exchange with a pH-dependent variable stoichiometry has implications for the operation of the carrier at a molecular level. A model that accommodates the functional properties of the antiporter assumes that the protein possesses a bifunctional active site that binds either monovalent species independently or divalent species co-operatively (Maloney *et al.*, 1990). Although the *uhpT* transporter of *E. coli* is functional as a monomer (Ambudkar *et al.*, 1990), the substructure of the protein, resembling that of a dimer, would be compatible with the model (Maloney *et al.*, 1990).

The significance of catalysing sugar 6-phosphate/phosphate, *sn*-glycerol 3-phosphate/phosphate or PEP/phosphate exchange is not quite understood since sugar phosphates are not usually present in the bacterial environment. At the same time, cells may need to avoid leakage of these energy-rich intermediates. For *E. coli* and *S. typhimurium*, however, sugar phosphate uptake coupled to phosphate excretion could have some relevance in habitats like the intestinal tract of warm-blooded animals, in which pathway intermediates can be found (Starr *et al.*, 1981). In lactic acid bacteria, the reverse reaction, i.e. phosphate uptake at the expense of sugar phosphate excretion, has been implicated as a defence mechanism against unregulated sugar phosphate production, which can be bactericidal (Thompson and Chassy, 1982).

A system catalysing phosphate self-exchange has been identified in *Streptococcus pyogenes* (Reizer and Saier, 1987). At present, no physiological role can be assigned to this transport system since substrates other than phosphate (arsenate) have not been found.

Lactose/galactose antiport

Streptococcus thermophilus and *Lactobacillus bulgaricus* transport lactose by means of a secondary transport mechanism after which the disaccharide is hydrolysed into glucose and galactose by β-galactosidase (Poolman *et al.*, 1989, 1990; Schmidt *et al.*, 1989). Glucose enters the glycolytic pathway, whereas in the presence of excess lactose, the galactose moiety of lactose is excreted into the medium stoichiometrically (Thomas and Crow, 1984). The excretion into the medium of the galactose moiety of lactose and the apparent *gal*$^-$ phenotype of *S. thermophilus* have been attributed to a defect in the induction mechanism for galactokinase (Hutkins *et al.*, 1985). The uptake of lactose and the excretion of galactose could occur in symport with proton(s) (Fig. 1C, upper part).

The lactose transport genes (*lacS*) of *S. thermophilus* (and *L. bulgaricus*) have been cloned, sequenced and expressed functionally in *E. coli* (Poolman *et al.*, 1989, 1990; B. Poolman, S. Yoast, S. E. Mainzer, and B. F. Schmidt, manuscript in preparation). When expressed in

E. coli, the lactose transport protein catalyses uphill transport in apparent response to a Δp. Not only lactose but also galactose and the non-metabolizable β-galactoside analogue methyl-β-D-thiogalactopyranoside (TMG) are transported efficiently by the *lacS* protein. Preliminary experiments suggest that during lactose metabolism in *S. thermophilus* lactose uptake and galactose excretion are intimately coupled, i.e. they proceed as a lactose/galactose antiport (Fig. 1C, lower part). In this pathway no metabolic energy is spent for the uptake of lactose and/or the excretion of galactose. The evidence is the following: (i) exit of galactoside from resting cells can be stimulated almost 100-fold when galactoside (e.g. TMG) is present externally; and (ii) rates of TMG uptake in the presence of a metabolizable substrate (e.g. sucrose or glucose) are too low to account for the observed rates of lactose metabolism whereas rates of TMG/TMG or TMG/galactose exchange are in accordance with the maximum rates of lactose utilization.

These data imply that functional expression of the galactokinase gene (*galK*) would lead to a decreased rate of lactose uptake since galactose would no longer be available for the antiport reaction. Although the organism would be able to utilize both sugar moieties, the decreased rates of lactose metabolism could be a selective disadvantage in media containing an excess of the disaccharide. In fact, *galK*$^+$ strains of *S. thermophilus*, selected in lactose-limited chemostats at low dilution rates, are very unstable and lose their ability to utilize galactose rapidly upon transfer to media containing an excess of lactose (Thomas and Crow, 1984). The kinetic advantage of lactose/galactose antiport can also be inferred from observations that natural *gal*$^+$ strains, e.g. *S. thermophilus* type strain ATCC 19258, acidify milk slowly compared to *gal*$^-$ (*galK*) strains (C. J. Schroeder, C. Robert, G. Lenzen, L. L. McKay, and A. Mercenier, submitted for publication).

Some lactic acid bacteria excrete fructose when metabolizing sucrose. The basis for transport of these sugars could be sucrose/fructose antiport similar to lactose/galactose antiport (Thomas and Crow, 1983).

Oxalate/formate antiport

Oxalate serves as the sole source of metabolic energy for growth of *Oxalobacter formigenes* (Allison *et al.*, 1985). Since oxalate is converted into formate and carbon dioxide, the generation of metabolic energy from oxalate must occur during oxalate uptake and formate excretion or as a result of decarboxylation. Since the decarboxylase of *O. formigenes* is a soluble enzyme (Baetz and Allison, 1989), coupling of decarboxylation to sodium (cation) extrusion seemed less likely (Dimroth, 1987). Therefore, it was hypothesized that the transport of oxalate contributes to energy conservation in *O. formigenes* by electrogenic exchange of divalent oxalate for monovalent formate (Anantharam *et al.*, 1989). In this scheme, entry of a divalent oxalate is followed by its decarboxylation in a reaction that consumes a proton to yield formate and carbon dioxide. Consequently, the exchange of oxalate for formate in combination with the intracellular decarboxylation yields a net energy gain of one H^+/turnover (or 1/3 ATP equivalent/turnover, given a stoichiometry of $3H^+$/ATP for ATP synthesis by an F_0F_1-ATPase). Experimental evidence for electrogenic oxalate/formate antiport in *O. formigenes* has been obtained by using proteoliposomes (Anantharam *et al.*, 1989).

Malate/lactate antiport

Malolactic fermentation can be carried out by species of *Leuconostoc, Lactobacillus, Lactococcus* and *Pediococcus* (Cox and Henick-Kling, 1989; Pilone and Kunkee, 1972; Renault *et al.*, 1988). In this pathway malate enters the cells and is decarboxylated by malolactic enzyme to yield lactate and carbon dioxide, after which the lactate and carbon dioxide exit. The decarboxylation of malate by malolactic enzyme is a single non-energy-yielding step (Renault *et al.*, 1988). Since catabolism of malate by *Leuconostoc oenos* and *Lactobacillus plantarum* increases the intracellular pH and the membrane potential, it has been proposed that the electrogenic efflux of lactate and/or carbon dioxide is responsible for the metabolic energy produced (Cox and Henick-Kling, 1989). Alternatively, one might propose that an electrogenic malate/lactate antiport is operative in these organisms. Uptake of divalent malate followed by its decarboxylation in a reaction that consumes one H^+, and excretion of monovalent lactate would yield a net energy gain of one H^+/malate metabolized (Fig. 1D). This biochemical cycle is analogous to the one for oxalate catabolism in *O. formigenes*. In line with the low pH at which malolactic fermentation is operative (Renault *et al.*, 1988) and energetically equivalent to malate^{2-}/lactate^{1-} antiport, the exchange reaction could also be malate^{1-}/lactic acid. Evidence supporting malate/lactate antiport in malolactic organisms has been recently obtained with *L. lactis* IL1403 (B. Poolman, E. J. Smid, T. Abee and W. N. Konings, unpublished results). By analogy with mitochondria, malate/phosphate antiport also may be found in (lactic acid) bacteria.

*Betaine/*N, N-*dimethylglycine antiport*

Betaine (*N,N,N*-trimethylglycine) is demethylated to *N,N*-dimethylglycine by various anaerobic bacteria (Heijthuijsen and Hansen, 1989; Muller *et al.*, 1981). The end-product, *N,N*-dimethylglycine, is excreted into the medium. The fate of the methyl group depends on the

species that metabolizes the betaine. In *Eubacterium limosum*, the methyl groups are used to form fatty acids (Muller *et al.*, 1981), whereas in a *Desulfobacterium* strain the methyl groups are oxidized to carbon dioxide and the reducing equivalents produced are used to reduce sulphate to sulphide (Heijthuijsen and Hansen, 1989). Since betaine and *N,N*-dimethylglycine are structurally very similar and the conversion is stoichiometric, one might speculate that transport of these solutes also occurs via antiport (Fig. 1E). Betaine/*N,N*-dimethylglycine antiport would represent a new class of exchange driven by the concentration gradients for betaine and *N,N*-dimethylglycine, and, because of the difference in charge of the solutes, by the membrane potential.

Conclusions

In this paper, metabolite transport by means of antiport has been reviewed. For arginine/ornithine, agmatine/putrescine, sugar 6-phosphate/phosphate and oxalate/formate antiport, metabolite exchange seems to be the mechanistic event. The Δp or one of its components can affect the translocation process through (de)protonation of the substrate(s) (see sugar 6-phosphate/phosphate antiport) or through the differential charge of the individual substrates (see oxalate/formate antiport). In other cases, antiport may occur on top of an H^+-symport reaction (see lactose/galactose antiport). Depending on the concentrations of lactose, galactose and protons on either side of the membrane, the affinity constants for these molecules and the magnitude of the membrane potential, uptake of lactose can be by means of lactose/galactose antiport or lactose/H^+ symport. Since galactose has to be excreted by *S. thermophilus* (and *L. bulgaricus*), lactose/galactose antiport seems to be most relevant under physiological conditions.

In a number of cases, the postulated precursor/product antiport has been inspired by energetic considerations, i.e. arginine/ornithine and agmatine/putrescine antiport (one mole of ATP is synthesized per mole of arginine or agmatine catabolized), oxalate/formate antiport (oxalate is the sole source of metabolic energy), malate/lactate antiport (malolactic fermentation yields ATP). In these examples, the energetic consequences of the proposed antiports in conjuction with the corresponding pathways are in accordance with observed growth yields. Furthermore, it can be assumed that the additional end-products carbon dioxide and ammonia diffuse outward without effect on the pH gradient.

Ignoring regulatory phenomena (Poolman *et al.*, 1987b; Konings *et al.*, 1989), the direction of transport via a secondary transport system is determined by the directionality of its driving force. If, in the case of Δp-driven solute transport, the outwardly directed concentration gradient of the solute exceeds the electrochemical gradient for protons (Δp), solute efflux will occur resulting in the conversion of the solute gradient into a Δp (see 'energy recycling model', Michels *et al.*, 1979; Ten Brink *et al.*, 1985). For the metabolic pathways discussed above, the energetic costs of precursor uptake could, in principle, be balanced by carrier-mediated product efflux. When the energy gain for the cell is similar, one may wonder whether a preference for precursor/product antiport with regard to precursor uptake and product efflux via separate systems still exists. Other advantages of an antiport mechanism can be recognized: (i) the uptake of precursor drives the excretion of product and vice versa; (ii) the linkage of precursor and product movements assures tight coupling to the corresponding metabolism, which minimizes (excessive) product accumulation and possible product inhibition. Furthermore, changes in the rates of metabolism are reflected directly in the rates of exchange through changes in the intracellular concentrations of precursor and/or product. (iii) Solute transport by means of exchange is usually much faster than transport via mechanisms with other modes of energy coupling (Kaback, 1983; Konings *et al.*, 1989; Maloney *et al.*, 1990).

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